CXC Chemokines and Their Receptors Are Expressed in Type II Cells and Upregulated following Lung Injury

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The proinflammatory CXC chemokines GRO, CINC-2α, and macrophage inflammatory protein (MIP)-2 are a closely related family of neutrophil chemoattractants. Here, we report that freshly isolated alveolar Type II (TII) cells express these chemokine mRNAs at much higher levels than do freshly isolated Type I cells or alveolar macrophages (AM). TII cells also express CXCR2, the receptor for these chemokines. Lung injury caused by acid or *Pseudomonas aeruginosa* (*Pa*) caused an increase in TII cell expression of chemokine mRNAs and GRO protein. We compared the time courses of chemokine mRNA expression in cultured TII cells and AM. In TII cells, GRO mRNA levels were stable over 4 h, but decreased to undetectable levels by 24 h. CINC-2α and MIP-2 mRNA levels were low in freshly isolated cells, increased over 2–4 h in culture, and by 24 h dropped to undetectable levels. In contrast, none of these chemokine mRNAs were detected in freshly isolated AM, but expression was induced by tissue culture. In summary, we have shown that TII alveolar epithelial cells produce three of the major proinflammatory CXC chemokines (GRO, CINC-2α, and MIP-2) and their cognate receptor CXCR2. Chemokine expression is upregulated in response to lung injury. These observations support a central role for the TII cell as an immunologic effector cell in the alveolus and raise intriguing questions about how CXC chemokines and receptors modulate diverse normal and pathologic cellular responses in the alveoli.

In addition to its primary function in respiration, the lung has developed an efficient host defense network that requires regulated recruitment of inflammatory cells to sites of infection and injury. The primary mediators of inflammatory cell recruitment are the chemokines, small (10 kD), chemotactic cytokines that induce directional migration and activation of leukocytes (reviewed in Ref. 1). There are more than 50 distinct chemokines, broadly classified into C, CC, CXC, and CX_3C subgroups based on the arrangement of conserved cysteines located near the amino terminus of the protein. For example, in the CC family of chemokines, the cysteines are adjacent, whereas in the CXC chemokines, they are separated by a single nonconserved amino acid. This structural classification has a general, not absolute, functional correlate in that CC chemokines target monocytes, T cells, eosinophils, and basophils, whereas CXC chemokines target neutrophils (1).

A conserved glutamate-leucine-arginine sequence (ELR) near the amino terminus of certain CXC chemokines confers high-affinity binding to CXCR2, a G-protein–coupled chemokine receptor (2). The ELR+CXC chemokines are generally proinflammatory, with human interleukin (IL)-8 being the best characterized example. Rats and mice lack the IL-8 gene due to a deletion in a common ancestor (3). The "GRO-related" ELR+CXC chemokines, including macrophage inflammatory protein (MIP)-2 (4), CINC-2α (5), and GRO (6) itself, are functional IL-8 correlates in the rat, with similar characteristics of neutrophil chemoattractant properties (4, 7), conserved IL-8–like tertiary structure (3), and binding and activation of CXCR2 (8). CXCR2 knockout mice exhibit defective neutrophil chemotaxis, pathogen exposure–dependent defects in the myeloid and B-cell lineages, and defects in wound healing (9, 10).

Although initial concepts regarding functions of Type II (TII) cells and alveolar macrophages (AM) focused on the role that TII cells play in surfactant metabolism and the role of AM in mediating inflammatory responses, current evidence suggests that there is overlap in functions of these two cell types (for reviews, *see* Refs. 11–14). AM are involved in surfactant homeostasis (12) and TII cells both express immunoregulatory molecules and serve immunoregulatory functions. Surfactant proteins A and D, both produced by TII cells, are involved in both innate and adaptive responses to pathogens (reviewed in Ref. 11). TII cells produce various cytokines (15), CC chemokines (16), and immune regulatory molecules (17), but a detailed examination of the ELR+CXC chemokines and CXCR2 in these cells has not been reported.

Our interest in chemokines in TII cells was recently stimulated by the observation that, by differential display PCR, GRO was expressed by TII cells, but not by Type I (TI) cells or AM (18). GRO chemokine expression has been examined in infected bovine and rabbit lung tissue with different techniques and opposite conclusions. In one report, bovine TII cells were positive for GRO protein expression (19); in contrast, rabbit TII cells were negative for GRO by *in situ* hybridization (20). Standiford and coworkers (21) reported that A549 cells express IL-8 mRNA in response to treatment with tumor necrosis factor or IL-1β, but careful evaluation has shown that A549 cells do not

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Abbreviations: alveolar macrophages, AM; cytokine induced neutrophil chemoattractant, CINC; conserved glutamate-leucine-arginine sequence, ELR; growth related oncogene, GRO; interleukin, IL; macrophage inflammatory protein, MIP; *Pseudomonas aeruginosa*, *Pa*; surfactant protein, SP; alveolar type II cells, TII cells.

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display important morphologic (22), lipid (22), and molecular (23, 24) markers of the type II cell phenotype, raising the question of the comparability of these studies to TII cells. There is growing evidence that TII cells produce a range of chemokines, including MIP-2, in response to recognition by antigen-specific CD-8+ T cells (25, 26). In this communication, we characterize expression of GRO-related chemokines and CXCR2 in isolated rat alveolar epithelial and macrophage cell populations, as well as in normal and injured lung. Our data demonstrate that TII cells are not only a source of GRO-related chemokines within the lung, but also express the receptor for these chemokines. Furthermore, lung injury induces expression of this chemokine/receptor network in TII cells. These findings, taken together with other studies of immunoregulatory functions of TII cells, suggest that TII cells play a central role in modulating host defense in the distal lung.

Materials and Methods

Isolation and Culture of Cells

Isolation of alveolar Type I and TII cells from 300 g male Sprague-Dawley rats was performed as described previously (27) with the following exceptions for isolation of type II cells. All solutions were obtained from the UCSF Tissue Culture Facility and contained < 0.03 EU (0.015 ng) endotoxin/ml. Lungs were perfused via the vasculature with phosphate-buffered saline, then lavaged via the trachea with calcium- and magnesium-free phosphate-buffered saline containing 1 mM EDTA and 1 mM EGTA. AM were isolated by bronchoalveolar lavage using the same solution as that for lavage in the type II cell isolation procedure. The purity of cell preparations was assessed with antibodies to TI cell (28) and TII cell (29) integral apical membrane cell-specific antigens. Preparations of TI cells contained < 5% TII cells; preparations of TII cells contained < 1% TI cells. TII cells (3.75×10^6/6-cm dish) were cultured at 37°C, 10% CO_2 in DME-H16, 10% fetal bovine serum (Hyclone, USDA tested, containing < 25 EU endotoxin/ml) and 50 μg gentamicin/ml. For most of the experiments, type II cells were cultured on a substratum of tissue culture plastic. For experiments in which type II cells were cultured under conditions designed to preserve expression of the type II cell phenotype, cells were cultured on a substratum of EHS matrix (Matrigel, BD Biosciences, Bedford, MA) :DMEM (2:1) in DMEM containing 1% rat serum (Pel-Freeze, Rogers, AK), 100 μM 8 Bromo-cAMP (Sigma, St. Louis, MO), 10 ng KGF/ml (R&D Systems, Minneapolis, MN), 50 μg gentamicin/ml. Alveolar macrophages (5×10^6 cells/10-cm dish) were cultured at 37°C, 5% CO_2 in RPMI-1640, 10% fetal bovine serum, and 50 μg/ml gentamicin.

Probes

Primer pairs were used with alveolar macrophage RNA isolated from LPS-treated rats in RT-PCR reactions (Ambion Retroscript, Austin, TX; 55°C annealing temperature for 25 cycles) to generate probe fragments specific to each GRO-family member, based on GenBank sequence data. The nucleotide sequences are as follows: GRO (Acc. # D11444.1), nucleotides (inclusive) 231–684; CINC-2α (D87926.1), 1484–1781; MIP-2 (U45965.1), 431–788.

These probes, each from the 3′-UTR of the respective cDNA sequence, do not crossreact with one another; therefore, they permit specific chemokine mRNA detection in Northern blotting. PCR products were cloned directly into pCR-II TOPO (Invitrogen, Carlsbad, CA) and the inserts sequenced to confirm their identity.

Northern Blots

Total RNA, isolated with RNA-Stat 60 (Tel-Test, Friendswood, TX), was resolved on agarose-formaldehyde gels, then transferred and ultraviolet-crosslinked to nylon membranes. Blots were hybridized in ExpressHyb solution (Clontech, Palo Alto, CA) with ^{32}P-labeled, randomly primed (Invitrogen) probes and washed, all as recommended by the vendors. Final washes were in 0.1× SSC/0.1% SDS at 50°C. Blots were analyzed on a phosphorimager and exposed to X-ray film. Blots were stripped by boiling in 0.05% SDS for 10 min followed by phosphorimaging to confirm probe removal. For RNA loading controls, each blot was hybridized in probe excess with a low specific activity 18S rRNA riboprobe (Ambion Megascript).

Rat Lung Injury

Male Sprague-Dawley rats (300–350 g; Charles River Laboratories, Wilmington, MA), certified pathogen free, were used in the experiments. All animal experiments were done in compliance with the Animal Care Committee and Biosafety Committee of the University of California at San Francisco. For acid injury, instillates were prepared from hydrochloric solution (pH 1.15, 0.1N, endotoxin-free; Sigma Chemicals, Co., St. Louis, MO) mixed with normal saline (normal saline:hydrochloric acid ratio was 1:2) (30). For bacterial injury, the cytotoxic *Pseudomonas aeruginosa* (*Pa*) strain PA103 was used (31). PA103 was subcultured on Vogel-Bonner minimal medium and inoculated into trypticase soy broth containing 10 mM nitrilotriacetic acid. The bacterial pellet was washed and diluted into the appropriate number of colony-forming units per milliliter in lactated Ringer's solution as determined by spectrophotometry. The number of bacteria was confirmed by plating dilutions of bacteria on sheep blood agar plates. For the tracheal instillation, rats were anesthetized with inhaled sevoflurane (Ultane; Abbott Laboratories, Abbott Park, IL); once anesthetized, they were placed supine. A blunt end–modified needle (18 gauge) was inserted into the trachea, and 1.0 ml of the instillate (either acid, bacterial instillate, or normal saline) was administered using a syringe. The rats were monitored for coughing and regurgitation, allowed to recover from the inhalation anesthetic, and were returned to their cages. After 4 h, they were killed with 50 mg pentobarbital sodium/kg and lungs were excised for analyses.

In some experiments, rats were depleted of AM by aerosolization of liposomes containing disodium clodronate (31, 32) before injury. Based on previously published data using identical methods, we would expect ~ 95% percent of AM to be depleted.

Immunohistochemistry

Rat lung tissue (33, 34) and cytocentrifuged preparations of cells (33) were fixed and prepared for immunocytochemistry using antigen retrieval as described previously. Primary rabbit antibodies against rat GRO (cat# 500-P74; PeproTech, Rocky Hill, NJ) and mouse CXCR2 (cat# sc683, Santa Cruz Biotechnology, Santa Cruz, CA) were detected using Alexa 488-goat anti-rabbit IgG (Molecular Probes, Eugene, OR) as a secondary antibody. We used an anti-RTII70 monoclonal antibody (29) followed by Alexa 594-goat anti-mouse IgG (Molecular Probes, Eugene, OR) to label TII cells.

Results

Expression of GRO Protein in Freshly Isolated and Cultured TII Cells

Results of differential display PCR experiments indicated that GRO was preferentially expressed in freshly isolated TII alveolar epithelial cells, compared with freshly isolated

TI cells and AM (18; E. Mager, unpublished observations). We used a commercial anti-GRO antibody to investigate expression of the chemokine in freshly isolated TII cells (Figure 1). Cytocentrifuged preparations of isolated cells were doubly stained with two different primary antibodies and isotype-specific secondary antibodies conjugated to different fluorophores. RTII70 (29), an integral membrane protein specific within the lung to TII cells, is indicated by a red signal, GRO by a green signal. TII cells were heterogeneous in the amount of expression of GRO that was detectable by immunofluorescence; many TII cells appeared to be negative.

Preferential Expression of GRO-related Chemokine mRNAs in Isolated TII Cells, Compared with Isolated TI Cells and Alveolar Macrophages

The three rat ELR+CXC chemokines GRO, CINC-2α, and MIP-2 are encoded by 1.3 kb mRNAs that exhibit ~ 90% nucleotide identity within their respective coding regions (4, 5, 7). We designed a set of three highly specific, 3′-UTR cDNA probes to analyze expression of these chemokine genes in TI cells, TII cells, and AM. A Northern blot of RNA from these cell populations was sequentially hybridized with the chemokine probes (Figure 2). Of the three mRNAs, all were enriched in TII cells; the mRNA for GRO appeared to be more abundant than that for CINC-2α or MIP-2. None of these three chemokine mRNAs was detected in freshly isolated AM (Figures 2 and 3). The isolated TII cells were > 90% pure; the TI cell preparation was ~ 80% pure. Preparations of TI cells contained less than 5% TII cells; preparations of TII cells contained less than 1% TI cells. There were no TI or TII cells present in the preparations of AM. Given these levels of purity in the cell populations and the results of the Northern blots shown in Figure 2, we conclude that TII cells preferentially express all three GRO related chemokine genes, in contrast to TI cells and freshly isolated AM.

Induction of GRO-Related Chemokine mRNA Expression in Cultured AM

We consistently observed ($n = 4$) that freshly isolated AM do not contain mRNAs for the GRO-related chemokines by Northern blot analysis. The lack of GRO-related CXC chemokine expression in freshly isolated AM was surprising because early studies of these molecules involved macrophages (4, 5). However, these studies used macrophages maintained in tissue culture, either from peritoneal (5) or alveolar (4) sources, whereas the macrophages used for our experiments were not cultured. To determine the effects of tissue culture on chemokine expression in macrophages, we investigated chemokine gene expression in AM as a function of time in culture (Figure 3A). Northern blots were prepared with RNA from AM cultured for 0, 2, 4, 8, and 24 h. Consistent with the lack of signal in freshly isolated AM (Figure 2), none of the GRO-related chemokine mRNAs were detected in the time 0 (freshly isolated) cells. By 2 h of culture, GRO and MIP-2 mRNA levels were dramatically induced, whereas CINC-2α remained undetectable. GRO and MIP-2 mRNA levels gradually fell to very low levels between 4 and 24 h in culture, whereas

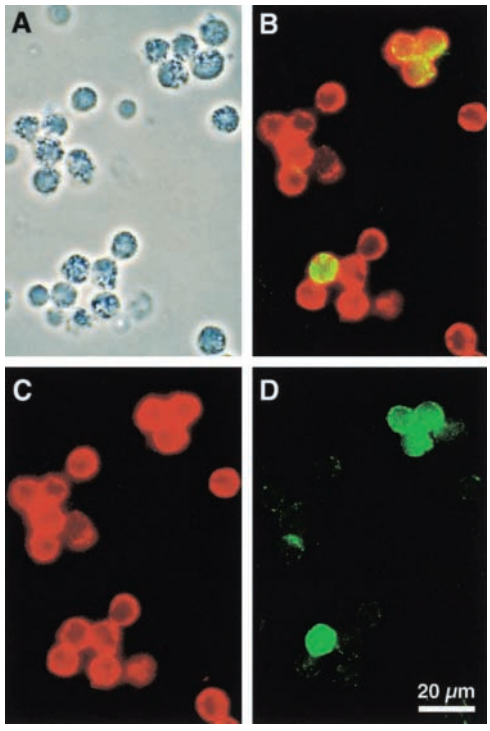

Figure 1. Expression of GRO protein in isolated TII cells. Cytocentrifuged preparations of isolated TII cells were probed with two primary antibodies, a rabbit anti-rat GRO and a mouse monoclonal anti-rat RTII70 (TII cell–specific protein), and then stained with appropriate specific secondary antibodies conjugated to fluorophores, as described in Materials and Methods. Isolated TII cells (*red*) were heterogeneous in whether they contained GRO protein (*green*) and in the amount of GRO that was detectable by immunofluorescence; some cells were weakly positive, others were strongly positive, and many were negative. The following panels show matching phase contrast and immunofluorescence images: *A*, phase contrast; *B*, merged image of cells shown in *C* and *D*, showing TII cells expressing GRO protein in *yellow* (addition of *green* and *red* colors); *C*, anti-TII70 (*red*); *D*, anti-GRO (*green*).

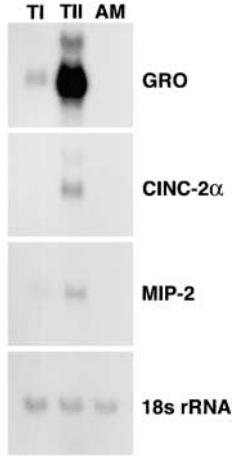

Figure 2. Expression of rat GRO chemokine family mRNAs in alveolar cells. A Northern blot of RNA prepared from isolated rat alveolar TI cells (TI), TII cells (TII), and macrophages (AM) was sequentially hybridized with the indicated chemokine-specific probes as described in Materials and Methods. The preparation of TI cells contained a small (< 5%) number of TII cells. The blot was also probed with a very low specific activity 18S rRNA probe as a loading control. Chemokine expression is prominent in TII cells and essentially negative in isolated AM.

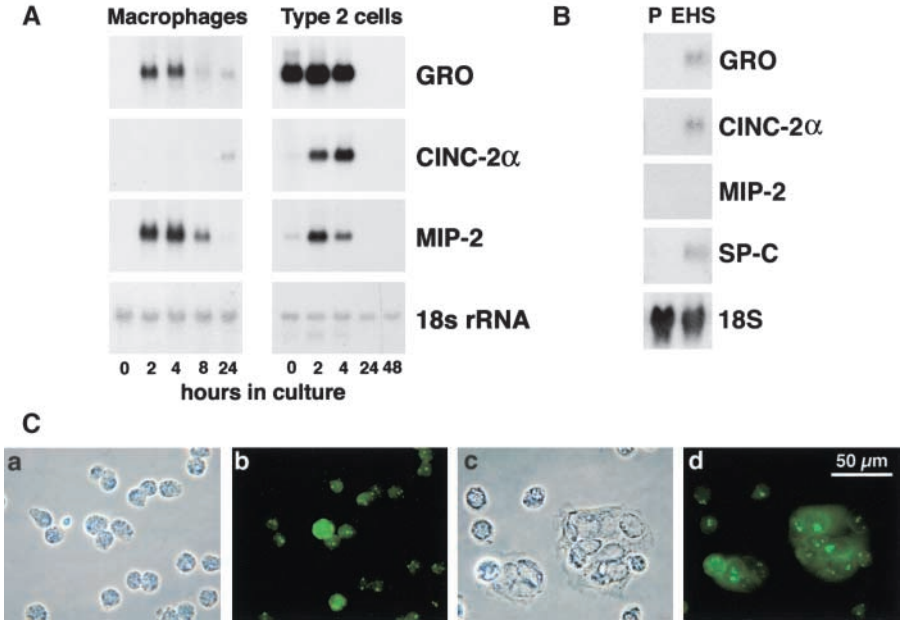

Figure 3. Time course of GRO chemokine family mRNA and protein in cultured AM and TII cells. (*A*) Northern blots, prepared with RNA from cells cultured on tissue culture plastic for the indicated period of time, were sequentially analyzed with the indicated CXC chemokine probes. Freshly isolated macrophages, in contrast with TII cells, did not express mRNAs for this family of chemokines. The time course of expression varied with cell type and with each specific chemokine. (*B*) Type II cells cultured for 7 d on tissue culture plastic did not express detectable CXC chemokine mRNAs. In contrast, cells cultured on EHS matrix, which promotes retention of the type II cell phenotype (expression of SP-C mRNA), also expressed mRNAs for GRO and CINC-2α. (*C*) After 2 h (a, b) and 24 h (c, d) in culture, most type II cells express GRO protein; paired phase contrast (a, c) and fluorescence (b, d) images of type II cells cultured on plastic are shown.

CINC-2α mRNA first appeared during this interval, albeit at a low level compared with that of TII cells (Figure 2, and *see below*). Thus, cell culture activates GRO, MIP-2, and CINC-2α chemokine gene expression in AM.

Expression of GRO-Related Chemokine mRNA and Protein in Cultured TII Cells

We also investigated GRO-related chemokine mRNA and protein expression in cultured TII cells (Figure 3A). When cultured on plastic, type II cells cease to express markers of the TII cell phenotype (35). In Figure 3A, we show a similar change in expression pattern for the GRO-related chemokine genes. Between 4 and 24 h in culture, expression of all three GRO-related mRNAs falls to undetectable levels. Expression remains undetectable for a week in culture. In contrast (Figure 3B), when type II cells are cultured on a matrix enriched in laminin and type IV collagen in the presence of KGF, expression of GRO and CINC-2α mRNAs are maintained, as is SP-C mRNA, a marker of the type II cell phenotype. Of interest, mRNA for MIP-2 is not expressed under these conditions. These results suggest that expression of GRO mRNA may be part of the "normal" type II cell molecular phenotype.

GRO protein in cultured cells (Figure 3C) was evaluated by immunocytochemistry. By 2 h of culture on plastic, virtually all type II cells expressed GRO protein, although the intensity of the immunofluorescent reaction appeared to be heterogeneous. Expression of GRO protein persisted for up to 24 h in culture (Figure 3C). These results are consistent with the studies of injured lung *in vivo*, in which GRO protein expression in type II cells was upregulated after 4 h of injury (*see below*).

TII Cells Display Increased GRO Protein following Lung Injury

GRO protein was expressed at extremely low levels in TII cells of the intact lung (Figures 4A and 4D). To examine the effect of lung injury on GRO expression, we analyzed TII cell expression of GRO-related chemokines in two well-studied models of lung injury, acid instillation (30), and *Pa* infection (31). Four hours after injury by either acid or *Pa*, GRO protein was easily detected in TII cells (Figure 4).

Lung Injury in Macrophage-Depleted Lungs Promotes TII Cell Production of GRO-Related Chemokine mRNAs

Because we were interested primarily in TII cell chemokine expression, we sought to minimize the macrophage contribution to total chemokine production by depleting the lungs of macrophages before lung injury. We analyzed expression of the GRO-related chemokine mRNAs in both tissue and TII cells isolated from these lungs (Figure 5). None of the mRNAs were detected in control, uninjured lung (Figure 5, *lanes 1* and *2*), consistent with low level of GRO protein expression in normal lung observed by immunocytochemistry (Figures 4A and 4D). Acid-induced lung injury (Figure 5, *lanes 5* and *6*) caused a slight increase in GRO and CINC-2α mRNA levels, although MIP-2 remained undetectable. *Pa* infection (Figure 5, *lanes 7* and *8*) caused a dramatic induction of all three mRNAs. Because these Northern blots reflect mRNA abundance in total lung RNA, including inflammatory cells, we also performed the analysis on RNA from TII cells isolated from AM-depleted, *Pa*-infected lungs (Figure 5, *right-hand panels*). Bacterial infection dramatically induced expression of all three chemokine mRNAs in isolated TII cells. The levels in TII cells from the uninjured controls were similar to those shown in Figures 2 and 3; however, we could not detect expression in lung. These observations support the hypothesis that lung injury induces GRO-related chemokine expression in TII cells.

TII Cells Express CXCR2

The GRO-related chemokines bind specifically to CXCR2, a seven transmembrane domain cell surface receptor (8, 36).

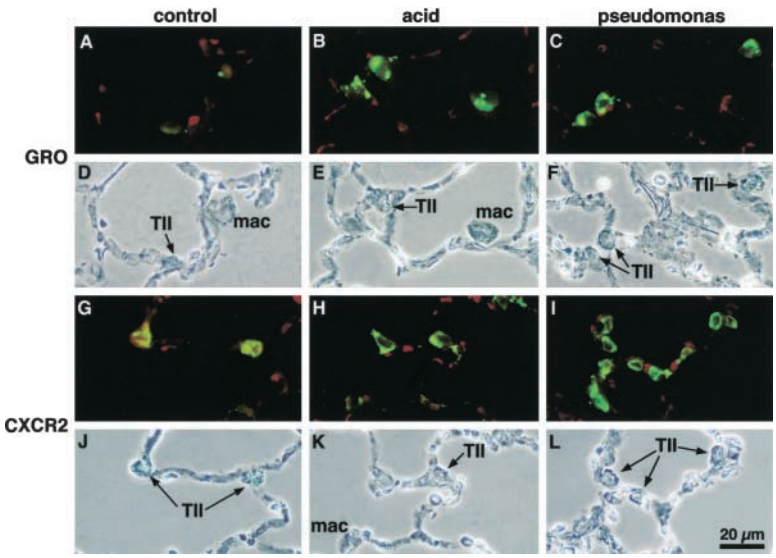

Figure 4. Expression of GRO and CXCR2 proteins in control and injured lung. Control (*A*, *D*, *G*, *J*), acid instilled (*B*, *E*, *H*, *K*), or *Pa*-infected (*C*, *F*, *I*, *L*) rat lung tissue was stained with anti-GRO (*A–C*) or anti-CXCR2 (*G–I*) antibodies and appropriate fluorescent secondary antibodies. Paired fluorescence (*A–C*, *G–I*) and phase contrast (*D–F*, *J–L*) images of distal lung parenchyma are shown. Lung injury upregulates expression of GRO.

Immunofluorescence with a commercial anti-CXCR2 antibody confirmed expression of CXCR2 in isolated TII cells (Figure 6). Expression of CXCR2 appeared more uniform than GRO expression (Compare Figure 6 and Figures 1, 4A, and 4G).

Discussion

We have shown (Figure 2) that freshly isolated rat alveolar TII cells express mRNAs for the GRO-related family of proinflammatory chemokines including GRO, CINC-2α, and MIP-2. Expression of GRO protein in these cells (Figure 1) is heterogeneous by immunocytochemical techniques. The heterogeneous expression may be due in part to limitations in detecting GRO protein by immunocytochemistry; techniques of antigen retrieval were used and it is possible that apparent differences in expression among type II cells are a result of being close to the threshold level of detection. Most, if not all TII cells, both *in situ* (Figure 4) and in isolated cells (Figure 6), express CXCR2, the

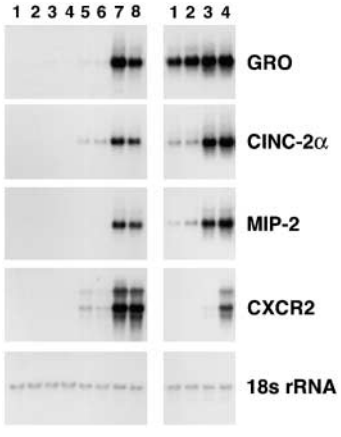

Figure 5. GRO-related chemokine mRNA levels in injured lung tissue and in TII cells isolated from the injured lungs. *Left panel*, *lanes 1–8:* Northern blots of total lung RNA probed with the indicated chemokine probe. Each lane contains 10 μg of RNA from a different rat lung or TII cells from different rats. Two rats in each group were treated as follows: *lanes 1* and *2*, no treatment; *lanes 3* and *4*, lungs were instilled with saline (vehicle control); *lanes 5* and *6*, lungs were instilled with a 0.1 N HCl solution in endotoxin-free saline; *lanes 7* and *8*, rats were infected with *Pa* (31). *Right panel*, *lanes 1–4:* Northern blots of RNA from TII cells isolated from lungs of macrophage-depleted rats, both control (*lanes 1* and *2*) and *Pa*-infected (*lanes 3* and *4*). Lung injury caused by either acid or Pseudomonas upregulates expression of chemokine mRNAs.

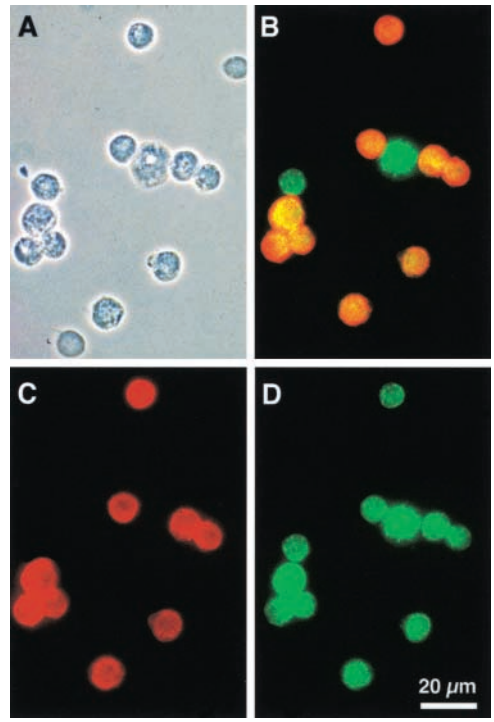

Figure 6. Expression of CXCR2 protein in TII cells. Cytocentrifuged preparations of TII cells were doubly stained with anti-TII70 and anti-CXCR2 antibodies as described in Materials and Methods. (*A*) Phase contrast. (*B*) Merged image of panels shown in *C* and *D* showing CXCR2-expressing TII cells in *yellow* (summation of *red* and *green* colors). (*C*) Anti-TII70 showing TII cells (*red*). (*D*) Anti-CXCR2 (*green*). All TII cells and two non-TII cells in the image express CXCR2.

high-affinity receptor for all three chemokines examined. Chemokine mRNA and protein levels increased in TII cells 4 h after lung injury caused by acid instillation or *Pa* infection (Figures 4 and 5).

The GRO-related chemokine mRNAs are ~1.3 kb long and share over 90% nucleotide identity within their coding regions. Any of these three cDNAs would likely cross-hybridize with the other two chemokine mRNAs on Northern blots. Our set of three probes, each from the 3′UTR of the respective cDNA sequence, allows specific chemokine mRNA detection on a Northern blot; there is no crossreactivity between the three probes (data not shown). The CINC-2α probe does not detect CINC-2β, an alternately spliced CINC-2 gene transcript which differs from CINC-2α in the C-terminal 3 amino acid codons and 3′UTR (5, 37). A CINC-2β 3′UTR region probe detected a number of lung-specific mRNAs (data not shown) including one that encoded rat lungkine, a CXC chemokine previously described in mouse upper airway tissue (38). Therefore, we could not reliably examine CINC-2β on Northern blots but could detect the transcript with RT-PCR as originally specified by Shibata and colleagues (37). We are currently studying the relationship between lungkine and CINC-2 transcripts in more detail.

The contrast between high-level GRO mRNA content in freshly isolated TII cells and the low-level expression in whole lung is striking. Although differences in the techniques preclude quantitative comparisons, GRO expression in TII cells *in situ* by immunocytochemistry appeared to be weaker and somewhat variable. At the mRNA level, freshly isolated TII cells expressed GRO abundantly, but in whole lung RNA we observed little if any GRO signal. In intestinal epithelium, GRO mRNA levels increase quickly in response to bacterial antigen exposure via a pronounced stabilization of the GRO mRNA (39). Based on these observations, we consider it likely that GRO and, by extension, CINC-2α and MIP-2, accumulate as a consequence of the TII cell isolation procedure *per se*. This procedure includes elastase digestion, tissue mincing, and cell filtration that may, in some way, reproduce some conditions of lung injury that also induce GRO. Of interest, 4 h after injury induced *in vivo* by bacteria or acid, expression of GRO protein is upregulated in TII cells in the lung. Taken together, these observations suggest that, in TII cells, expression of GRO is "primed" and can be rapidly upregulated in response to various stimuli. The rapid induction of chemokine production is compatible with the concept that TII cells may be a first line of mobilizing defense in the lung.

The functions of the various chemokines examined in this report are not completely understood. The human orthologs of the rat genes GRO, MIP-2, and CINC-2α are GRO α, β, and γ, but it is unclear which rat and human genes should be paired within this group (3). This, coupled with the absence of IL-8 in rats, makes extension of rodent chemokine studies to human lung biology difficult. Nonetheless, the rat proteins possess potent proinflammatory activities that are frequently compared with human IL-8. All three rat chemokines are strong neutrophil chemoattractants of similar potency when tested *in vitro* (5). However, structural differences in the interactions of GROα, IL-8, and NAP-2 with CXCR2 may lead to differential activation of downstream signaling pathways and functional distinctions between the chemokines (40). Similarly, human GROα and IL-8 elicit different downstream effectors in an *in vivo* model of neutrophil infiltration (41). Additional studies have examined these chemokines in the context of specific injury or pathologic state. For example, AM are clearly a major source of GRO expression in *Pa*-infected lungs (31). Depletion of AM in this model caused reduced GRO in bronchoalveolar lavage, reduced neutrophil recruitment, and reduced lung injury. However, at later stages of the disease process, macrophage depletion caused impaired bacterial clearance, enhanced neutrophil retention at the site of infection, and ultimately accelerated death from pulmonary injury (31). Our results in this same model of macrophage depletion suggest that TII cells expressing high levels of proinflammatory CXC chemokines may act in concert with AM to regulate the inflammatory response in pulmonary tissue.

Several independent reports suggest that TII cells play an important role in regulating inflammatory responses in the distal lung (for reviews, *see* Refs. 11, 13). The intriguing results of Enelow and colleagues show that TII cells expressing a transgenic influenza virus HA antigen gene are targets, in the absence of viral infection, of adoptively transferred, HA-specific cytotoxic T cells (25, 26). There are at least two distinct outcomes for the targeted TII cells: secretion of chemokines at low effector cell numbers, and lysis at very high effector cell numbers. Release of chemokines, the macrophage chemoattractant MCP-1 in particular, induces a profound inflammatory response resulting in severe, sometimes lethal, lung injury. Conversely, surfactant protein (SP)-A, a TII cell product, can downregulate inflammatory responses by specifically dampening T cell responses (14). Mice with deletions of either the SP-A or SP-D gene display heightened susceptibility to infection and inflammation (11, 12). Thus, TII cells clearly have both pro- and anti-inflammatory capabilities. Taken together, these results suggest that there may be TII cell regulatory sensors that balance synthesis and secretion of proinflammatory chemokines with anti-inflammatory molecules, such as SP-A and SP-D.

Although CXCR2 functions to mediate both positive (9, 42) and negative (43) chemotactic responses in cells such as neutrophils and oligodendrocytes, CXCR2 also has important functions in mediating morphologic changes, angiogenesis, wound healing, and proliferation. Examination of these noninflammatory roles may establish which, if any, of these CXCR2-mediated effects are active in TII cells. For example, the GRO-related chemokines and CXCR2 have a stimulatory effect on wound healing (9). In acute lung injury, TII cells may produce GRO not only to recruit inflammatory cells, but also to repair the alveolar epithelium by stimulating growth and proliferation of nearby cells. Human endothelial cells express both CXCR1 and CXCR2 and respond to IL-8 with a pronounced retraction of the cell periphery from its neighbors, a response that may promote neutrophil migration into the tissue surrounding targeted capillaries (42). A similar process permitting inflammatory cell migration across the alveolar epithelium might be medi-

ated by CXCR2 expressed on TII cells. Both GRO and overexpression of CXCR2 stimulate cell proliferation in melanoma cells (44, 45), raising the question of whether CXCR2 binding may also stimulate the proliferation in TII cells that occurs in response to lung injury. It further raises the intriguing possibility that TII cell proliferation may result in part from an autocrine function. Studies on cultured cells treated with chemokines should prove helpful in assessing many of these possibilities.

In summary, we have shown that freshly isolated rat TII alveolar epithelial cells produce three of the major proinflammatory CXC chemokines, GRO, CINC-2α, and MIP-2, and their cognate receptor CXCR2. Expression of chemokines is upregulated in response to lung injury with hydrochloric acid or *Pa* infection. These observations further support a central role for the TII cell as an immunologic effector cell in the alveolus, and raise intriguing questions about how CXC chemokines and receptors modulate diverse normal and pathologic cellular responses in the alveoli.

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